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Ovarian cholinergic innervation during different reproductive phases in house rat (*Rattus rattus*)

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Autonomic nerves influence, partially or completely, the follicular recruitment, development, ovulation and luteinization in many mammalian species (Burden and Lawrence 1978, Sporrong *et al.* 1991). Most of the information is derived from laboratory rat and no work on such aspects has been carried out in wild rodents. Therefore, the present investigation, was conducted to determine histochemically the acetylcholinesterase positive nerves in the ovary of *Rattus rattus* during different stages of prepubertal development, oestrous cycle, pregnacy and postpartum period and establish its relationship with different reproductive stages. Furthermore, immature rats were also treated with reserpine and serotonin to determine their effect on ovarian cholinergic innervation.

Immature, cycling, pregnant and postpartum female house rats collected from poultry farms, and godowns were sacrificed and their ovaries were processed for the histochemical localization of acetylcholinesterase (AchE). In addition, 3 sets of experiments were conducted, using 8 immature rats with 3 rats serving as controls for each experiment. Two sets of experimental animals were injected intraperitoneally with reserpine and serotonin each (1mg/100g of body weight in 0.9% saline) and were sacrificed after 24 hr of treatment. One set of animals was given same dose and route of reserpine but were killed after 72 hr of treatment. Ovaries from all the groups were processed for the localization of AchE activity by direct thiocholine procedure (Karnovsky and Roots 1964). The enzyme active sites which appeared reddish brown were recorded.

The ovary of house rat *Rattus rattus* was moderately supplied with cholinergic nerves. During all stages, large bundles of cholinergic nerve fibres which stained intensely for acetylcholinesterase (AchE), entered the ovary at the hilus region along the ovarian artery and followed the branches of this blood vessel in the medulla,where they stained strongly for AchE. They extended along with these blood vessels to its terminations in cortex, showing moderate enzyme activity (Fig.1). Such arrangement of perivascular nerves suggested its influence on vasculature. Since nerves which innervated blood vessels were helpful in regulating blood flow (Burden and Lawrence 1978). Apart from perivascular position, AchE nerves were also arranged in theca externa of growing follicles, atretic follicles, wall of corpus luteum and endocrine interstitial gland cells (Figs 2-7). This can be regarded to participate in non-vascular functions. These nerves may also contribute to facilitate the process of follicular growth itself and its associated changes in steroidogenic activity (Schultea *et al.* 1992). Many immunofluorescent studies reported the presence of neurotransmitters and catecholamines in nerve fibres closely associated with theca externa of antral follicles, interstitial tissue and within the tunica adventitia of blood vessels, performing the regulation of many ovarian functions (Dissen *et al.* 1991).

The AchE activity in immature drats was absent around stage 1 follicles; weak around preantral (stage 2, 3, 4 and 5) and preantral atretic follicles. This showed that ovary becomes subjected to neural inputs in the process of follicular recruitment, maturation and selection. It is well established that development of ovarian innervation precedes the onset of folliculogenesis and occurs before follicles acquire responsiveness to gonadotropins (Mayerhofer *et al.* 1997).

Single intraperitoneal reserpine injection to immature rats resulted in no noticeable change in the AchE activity after 24 hr but weak AchE activity appeared around stage 1 follicles after 72 hr. This is in contrast to their known function for depleting catecholamines and inhibiting ovulation (Hopkins and Pincus 1964). Reserpine severely affects both large and fine meshwork of esterase activity in rat (Guraya *et al.* 1991) and chick (Parshad *et al.* 1993) ovary. Marked changes were recorded after serotonin treatment in cortex, and around follicles. Since serotonin localizes in nerve like structures in immature rat ovary (Ameta *et al.* 1992), it may be enhancing

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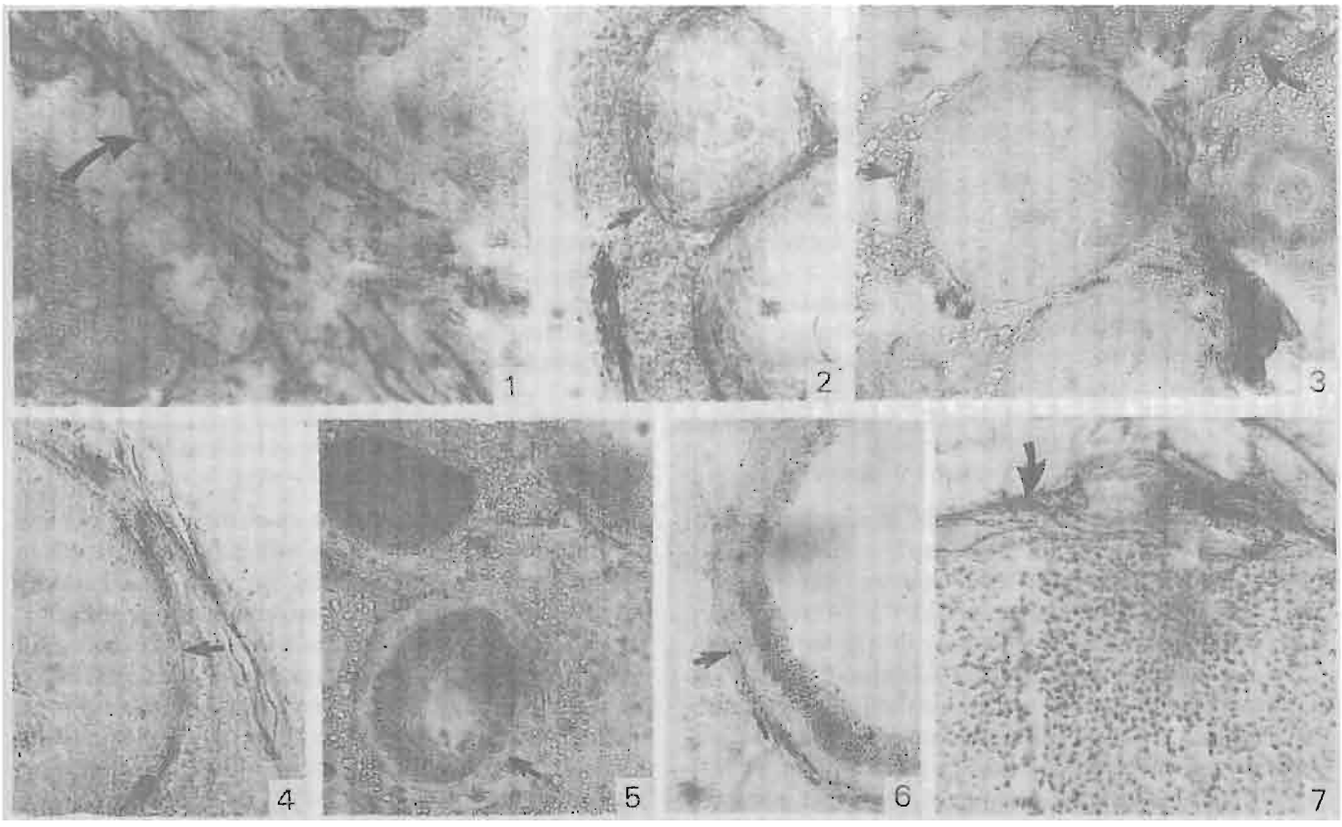

Figs 1-7. 1. Network of nerves in cortex of ovary (×100). 2. Distribution of AchE positive nerves around small developing follicles (×100). 3. Cholinergic nerves seen in the preantral follicle wall and around interstitial gland tissue (×100). 4. Antral follicle wall is encircled by AchE positive nerves (×100). 5. Weak AchE activity can be seen around atretic follicle (×100). 6. Antral atretic follicle showing moderate AchE reaction in its follicular wall (×100). 7. Rich distribution of cholinergic nerves can be seen in the wall of developed corpus luteum (×100).

the esterase activity within ovary, thereby influencing ovarian functions.

Intensity of AchE reaction increased in the cortex at proestrus stage whereas rest of the stages maintained moderate enzyme activity. Enzyme activity increased around stage 5 follicles at estrus. AchE positive sites were maximum in the Graafian follicles, which may be involved in the process of ovum expulsion. This process of ovulation depends on positive feedback actions of preovulatory estrogen secretions and specific neural signals for the initiation of LHRH surge (Levine 1997). The metestrus ovary revealed lack of enzyme activity around stage 1 to 3 and weak AchE reaction around antral follicles. This diminished activity could be correlated with decline in ovarian follicular growth at this stage. However, atretic follicles (preantral and antral) maintained weak and moderate AchE activity, respectively, during oestrous cycle. This AchE activity may be involved in thecal hypertrophy, luteinization and steroidogenesis by regulating haemodynamic changes (Guraya *et al.* 1991).

During pregnancy, stage 1 to 3 of normal follicles lacked AchE activity, and antral follicles (both normal and atretic) revealed weak AchE reaction from early to mid gestation period. However, the cortex became strongly positive for AchE in the advanced stages of pregnancy, with follicles showing moderate enzyme reaction. Such increase in density and intensity of interstitial adrenergic and cholinergic nerves were observed by Falck and Owman (1965) as pregnancy progressed. The ovarian norepinephrine content and progesterone production reportedly increased with advancement of pregnancy (Jordan *et al.* 1978). Rats in their lactation phase showed moderate activity of AchE around normal follicles but weak around atretic follicles.

Irrespective of stage of reproductive phase, developed corpora lutea possessed moderate AchE activity in their external layer while regressed corpus luteum revealed weak activity, which possibly suggested the involvement of AchE activity in maintaining blood vascularity by regulation of transport of luteotrophic and/or luteolytic factors. Present findings mainly characterize the qualitative distribution of cholinergic nerves and provide morphological data consistent with role of these nerves in ovarian functions.

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